Cytokinesis: **A regulatory role for Ras-related proteins?**

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Recent molecular genetic studies on the slime mold *Dictyostelium* have implicated Ras-related small GTP-binding proteins, and proteins that modify their activities such as Ras-GAPs, in the regulation of cytokinesis.

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Cytokinesis, the mechanism responsible for division of cytoplasm into two daughter cells at the end of mitosis, has intrigued biologists since it was first seen in the microscope well over a hundred years ago. Like most aspects of the cell cycle, cytokinesis is subject to precise controls, which determine both when it occurs and how the mother cell is spatially partitioned into the two daughter cells. Several recent papers [1–5], using molecular genetic approaches in the slime mold *Dictyostelium*, have provided important clues to the signaling pathways that control cytokinesis. These studies focus attention on the Ras-related small GTP-binding proteins and how they may regulate the state of the actin cytoskeleton, and perhaps other aspects of cytokinesis.

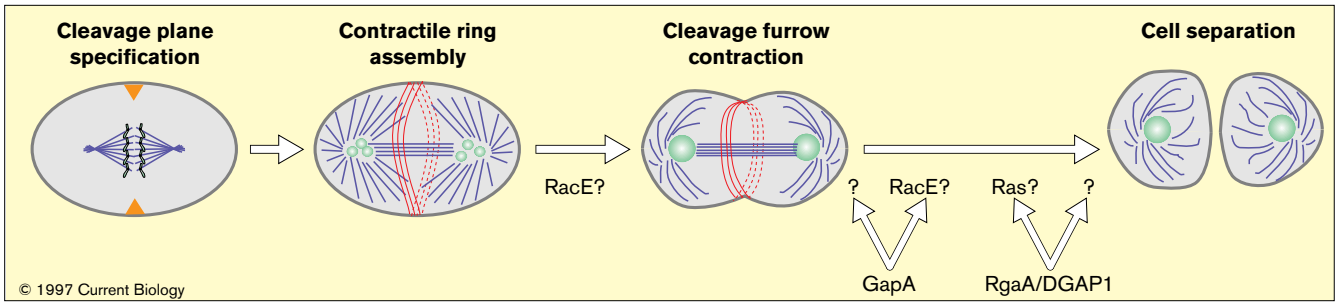
Cytokinesis consists of at least four separable steps (Figure 1; see [6] for a brief review). In the first step, the position of the cleavage plane is established. This positioning is clearly dependent on the orientation of the mitotic spindle. The molecular details of the process are unknown, but must involve a way of providing signals that direct the localized assembly of actin, the hallmark of the second step in cytokinesis — formation of the contractile ring. Ring assembly requires the concentration of actin at plasma membrane, where the furrow will form, followed by the subsequent localization of the major force generator for contraction, myosin II. The third step is the contraction of the cleavage furrow. Contraction most likely requires both activation of myosin, which presumably moves actin filaments past each other into a tighter and tighter 'purse string', and disassembly of the ring as the constriction between the future daughter cells decreases in size. The fourth and final step of cytokinesis is membrane fusion to complete the separation of the daughter cells; almost nothing is known about how this process occurs.

Two groups have identified genes required for cytokinesis in *Dictyostelium*, using the technique of 'restriction-enzyme-mediated integration' (REMI) [7]. In these experiments, transforming DNA was used as an insertional mutagen, and the resulting cells screened for defects in cytokinesis [3,8]. *Dictyostelium* has proven a particularly valuable system for studying cytokinesis, because the cells appear to have an alternative mechanism for cell division. This alternative process, known as traction-mediated cytofission, was first uncovered by mutations that removed myosin function [9,10]. These myosin mutant cells are unable to divide in suspension culture, forming gigantic cells with as many as fifty or more nuclei. Fortunately for those of us interested in cytokinesis, the mutants have normal growth rates on plastic surfaces, where the daughter cells are able to crawl away from each other with enough force to cause physical separation. This allowed Larochelle *et al.* [3,4] and Adachi *et al.* [8] to screen for mutations affecting genes important for cytokinesis, simply by looking for the formation of large multinucleate cells. It should be noted, however, that any gene required for both cytokinesis and traction-mediated cytofission would be missed by this screen.

From these screens, Larochelle *et al.* [3] identified a novel member of the Ras family, called RacE. Although cells lacking *racE* expression fail to grow in suspension and become multinucleate, they successfully complete the normal *Dictyostelium* developmental program and are able to cap receptors normally, both processes known to be dependent on acto-myosin function. As the small GTP-binding proteins Rac, Rho and Cdc42 have been implicated in regulating organization of the actin cytoskeleton, the normal organization of actin and myosin in the *racE* mutants suggests that RacE plays a very specific role in cytokinesis, rather than a more general role in actin cytoskeleton organization.

Larochelle *et al.* [4] have now reported a much more detailed analysis of the role RacE plays in cytokinesis. Starting with a *racE* mutant, the authors expressed either wild-type, constitutively-active or inactive mutant forms of RacE. Wild-type and constitutively-active RacE could both rescue the cytokinesis defect of the *racE* mutant; inactive RacE had no effect. This suggests that, while GTP-bound RacE can act in cytokinesis, it does not have to cycle between GTP-bound and GDP-bound states — arguing against RacE serving as a molecular switch responsible for the timing of cytokinesis. Furthermore, a fusion protein of RacE and the green fluorescent protein (RacE–GFP), which also rescued the cytokinesis defect of the *racE* mutant, was seen to be localized uniformly in association with the plasma membrane. This uniform localization was maintained even during cytokinesis, showing no concentration of RacE at the cleavage

Figure 1



The main steps in cytokinesis, indicating points at which small GTP-binding proteins may act. Actin is shown in red, microtubules in purple and the cleavage membrane in orange.

furrow. Moreover, as a constitutively active form of RacE that also localized uniformly over the entire plasma membrane failed to disrupt contractile ring formation, it seems unlikely that RacE specifies the site of contractile ring assembly. More recent results suggest that, in *racE* mutants, a contractile ring forms normally but fails to complete contraction (A. DeLozanne, personal communication). This suggests that RacE acts after assembly of the contractile ring.

The other REMI screen, performed by Adachi *et al.* [8] identified the *gapA* gene, which encodes a *Dictyostelium* homolog of Ras GTPase-activating protein (Ras-GAP). Two lines of evidence suggest that GapA acts very late in cytokinesis [1]. Unlike *racE* or *myosin II* mutants, *gapA* mutants are able to grow in suspension, albeit more slowly than wild-type cells. When the *gapA* mutant cells were videotaped during cytokinesis on a solid substrate, cleavage furrows were observed, often contracting to produce structures normally associated with the end of cytokinesis. These cleavage furrows showed a normal concentration of myosin, implying that both contractile ring assembly and contraction might be normal. These seem to fail before cytokinesis is completed, however, suggesting that GapA might activate a Rac or Rho family member required for completion of cytokinesis. For example, might GapA be required for the membrane fusion step required to separate the two daughters? Determining the subcellular localization of GapA protein might be very instructive in this regard.

Meanwhile, Lee *et al.* [2] carried out a two-hybrid screen using a constitutively active mammalian Ras as bait to fish for Ras-interacting proteins in *Dictyostelium*. In this way, they identified a second Ras-GAP, encoded by the *rgaA* gene. This gene had also been identified by Faix and Dittrich [11] — who called it *DGAP1* — using an antibody against a cell fraction enriched for actin-binding proteins. The *RgaA/DGAP1* and *gapA* products show 53% amino acid sequence identity and 64% similarity. Interestingly, both proteins show highest sequence similarity to human IQGAP, which has not only a GAP domain, but also 'IQ' domains, which have been implicated in binding calmodulin. GapA has a single IQ domain in a position corresponding to the IQ domains of human IQGAP. When *rgaA/DGAP1* was disrupted by gene targeting, the mutant cells grew poorly, if at all, in suspension, becoming large and multinucleate. Sound familiar?

That mutations in both *gapA* and *RgaA/DGAP1* cause cytokinesis defects suggests that, despite their similarity, they are not redundant and their products may act at distinct steps in cytokinesis. Furthermore, *rgaA* mutants grown in suspension become large multinucleate cells surrounded by small cells, which the authors suggest might be derived by 'budding' from the large cell. This may indicate that, like GapA, RgaA/DGAP1 acts after the contractile ring forms, most likely in the final stages of furrow contraction, furrow disassembly or membrane fusion.

In the most recent of the studies on small GTP-binding proteins and cytokinesis in *Dictyostelium*, Tuxworth *et al.* [5] knocked-out the *Dictyostelium rasG* gene. Guess what effect this had? Yes, the *rasG* mutant cells became large and multinucleate in suspension; they were generally less polarized than wild-type cells, suggesting a more generalized cytoskeletal defect than is the case for *racE* mutants. This is most likely because of the aberrant F-actin distribution caused by *rasG* disruption. The leading edges of the cells have spike-like, actin-rich filopods where wild-type cells would have broader pseudopods. In addition, punctate spots of actin are seen throughout the cytoplasm. This suggests that RasG may be involved in controlling the state of the actin cytoskeleton.

Video microscopy of *rasG* mutants undergoing cytokinesis showed apparently normal cleavage furrow formation, although it would be interesting to know more about the state of the actin in the contractile ring. Failure of cytokinesis appears to occur late in the process, as cells fail to separate completely, despite the fact that their cleavage

furrow appears to contract. This is the same stage of cytokinesis where the two Ras-GAP mutants, *gapA* and *rgaA*, fail. Might RasG be a target for GapA and/or RgaA/DGAP1? Might the abnormal aggregates of actin suggest a defect in disassembly of actin structures? If so, one interesting possibility is that RasG might facilitate the disassembly of the contractile ring.

Together, these recent studies present a compelling case that Ras family proteins play important roles in cytokinesis in *Dictyostelium*. The identification of signaling molecules as important players in cytokinesis, along with the characterization of Ras-GAP proteins that may regulate the GTP hydrolytic activity of these small GTPases, provides an outline of the signaling pathways that may be responsible for regulating cytokinesis. The tantalizing observation by Faix and Dittrich [11] that RgaA/DGAP1 may exist in association with actin fits nicely with the recent observation that a mammalian IQGAP not only binds directly to actin but can crosslink F-actin [12]. These results hint at some of the first molecular details of how cytokinesis might be regulated.

References

1. Adachi H, Takahashi Y, Hasebe T, Shirouzu M, Yokoyama S, Sutoh K.: **Dictyostelium IQGAP-related protein specifically involved in the completion of cytokinesis.** *J Cell Biol* 1997, **137:**891-898.
2. Lee S, Escalante R, Firtel RA: **A Ras GAP is essential for cytokinesis and spatial patterning in *Dictyostelium*.** *Development* 1997, **124:**983-996.
3. Larochelle DA, Vithalani KK, De Lozanne A: **A novel member of the rho family of small GTP-binding proteins is specifically required for cytokinesis.** *J Cell Biol* 1996, **133:**1321-1329.
4. Larochelle DA, Vithalani KK, De Lozanne A: **Role of *Dictyostelium* racE in cytokinesis: mutational analysis and localization studies by use of green fluorescent protein.** *Mol Biol Cell* 1997, **8:**935-944.
5. Tuxworth R, Cheetham J, Machesky L, Spiegelmann G, Weeks G, Insall R: ***Dictyostelium* RasG is required for normal motility and cytokinesis, but not growth.** *J Cell Biol* 1997, in press.
6. Glotzer M: **Cytokinesis.** *Curr Biol* 1997, **7:**R274-R276.
7. Kuspa A, Loomis W: **Tagging developmental genes in *Dictyostelium* by restriction enzyme-mediated integration of plasmid DNA.** *Proc Natl Acad Sci USA* 1992, **89:**8803-8807.
8. Adachi HH, Takeshi; Yoshinaga, Keisuke; Ohta, Takahisa; Sutoh, Kazuo: **Isolation of *Dictyostelium discoideum* cytokinesis mutants by restriction enzyme-mediated integration of the blasticidin S resistance marker.** *Biochem Biophys Res Commun* 1994, **205:**1808-1814.
9. DeLozanne A, Spudich JA: **Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination.** *Science* 1987, **236:**1086-1091.
10. Knecht DA, Loomis WF: **Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*.** *Science* 1987, **236:**1081-1086.
11. Faix J, Dittrich W: **DGAP1, a homologue of rasGTPase activating proteins that controls growth, cytokinesis, and development in Dictyostelium discoideum.** *FEBS Lett* 1996, **394:**251-257.
12. Bashour AM, Fullerton AT, Hart MJ, Bloom GS: **IQGAP1, a Rac- and Cdc42-binding protein, directly binds and cross-links microfilaments.** *J Cell Biol* 1997, **137:**1555-1566.